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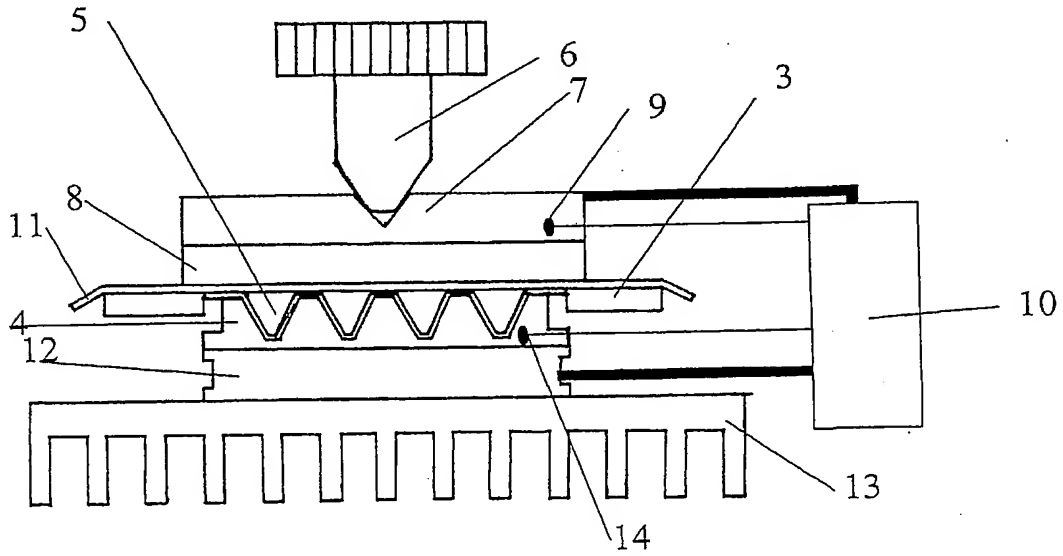
(54) Rapid heat block thermocycler

(57) A heat block thermocycler to perform rapid PCR in multiple small-volume samples (0.5-10 µl) employing a small, low-profile, low thermal capacity sample block the temperature of which can be rapidly and accurately modulated by a single thermoelectric pump. An array of spaced-apart sample wells is formed in the top surface of the block. The samples are placed into the wells of a small, ultrathin-walled (20-50 µm) multiwell plate and located into the sample block. The multiwell plate closely fits the array of sample wells and the top surface of the sample block. The heated lid tightly seals the wells by pressing the sealing film to the top surface of the multiwell plate supported by the top surface of the sample block. Air pressure arising inside the tightly sealed wells at elevated temperatures deforms the elastic walls of the wells of the ultrathin-walled plate and brings them into close thermal contact with the sample block. An elastic gasket thermally isolates the sample block from the heated lid.

Fig. 2



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Description

[0001] The invention relates to thermocyclers for an automatic performance of polymerase chain reaction (PCR), particularly to rapid thermocyclers. More specifically, it relates to rapid thermocyclers for parallel processing of multiple small-volume samples.
The present invention is especially useful for rapid, high-throughput, inexpensive and convenient PCR-based DNA-diagnostic assays.
Since its first published account in 1985 the polymerase chain reaction has been transformed into myriad array of methods and diagnostic assays. Temperature cycling of samples is the central moment in PCR. In recent years various rapid thermocyclers have been developed to address the slow processing speed and high sample volumes of conventional heat block thermocyclers. These rapid thermocyclers can be divided into two broad classes:

1. Capillary thermocyclers hold the samples within a glass capillary and supply heat convectively or conductively to the exterior of the capillary. For the description see Wittwer, C.T., et al., Anal.Biochem. 186; p328-331 (1990); Friedman, N.A., Meldrum, D. R. Anal. Chem., 70: 2997-3002 (1998) and U.S. Patent No 5,455,175.

2. Microfabricated thermocyclers are thermocyclers constructed of microfabricated components; these are generally etched structures in glass or silicon with heat supplied by integral resistive heating and rejected passively (or actively) to the ambient by the structure. However, other schemes of thermocycling, as continuous flow thermocycling of samples are also used. For the description see Northrup, M. A., et al., Transducers 1993: 924-926 (1993); Taylor, T.B., et al, Nucleic Acid Res., 25: pp 3164-3168 (1997); Kopp, M. U. et al., Science, 280: 1046-1048 (1998); U.S. Patent No 5,674,742; U.S. Patent No 5,716,842.

[0002] Both classes of rapid thermocyclers employ the increased surface-to-volume ratio of the reactors to increase the rate of heat transfer to small samples (1-10 µl). Total DNA amplification time is reduced to 10-30 minutes. Conventional heat block thermocyclers usually take 1-3 hours to complete temperature cycling of 10-100 µl volume samples. However, with these benefits also several disadvantages appear. The increased surface area between reagents and reactors (i.e. 10-80 fold compared to standard PCR tubes) causes a loss of enzyme activity, presumably due to electrostatic interactions between DNA polymerase and silanol anions that arise in silica during thermocycling. Furthermore, DNA can also be irreversibly adsorbed onto the silica surface of the reactors, especially in the presence of magnesium ions and detergents that are the standard components of a PCR mixture. Therefore, PCR in glass-silicon reactors requires the addition of carrier protein (e.g. bovine serum albumin) and a rigorous optimization of its concentration. Furthermore, the enzyme concentration has to be increased to obtain an equivalent performance to conventional PCR in plastic tubes.

[0003] Another disadvantage of these reactors is the very complicated way of loading and recovering the samples. In addition, the standard pipetting equipment is usually not compatible with such reactors. These inconvenient and cumbersome procedures are also time-consuming and labor-sensitive, thus limiting the throughput of the thermocyclers. Finally, although the reagents costs drop with a volume reduction to 1-10µl, the final costs are relatively high due to a high cost of capillary and, especially, microfabricated reactors.
The present invention bears some similarity to conventional heat block thermocyclers for performing PCR in plastic microplates (for example, see US Patent No 5,475,610). However, in contrast to conventional heat block thermocylers, it provides the means for rapid temperature cycling of small volume samples (i.e. 0.5-10 µl). More specifically, it provides a rapid heat block thermocycler for convenient, high-throughput and inexpensive, oil-free temperature cycling of multiple small-volume samples.

[0004] Accordingly, the invention concerns a heat block thermocycler for subjecting a plurality of samples to rapid thermal cycling, the heat block thermocycler comprising

- means for holding the plurality of samples comprising
  - the ultrathin-walled multiwell plate capable of holding a plurality of samples, and
  - a low profile, low temperature capacity sample block having an array of wells, wherein the said wells are of similar height and shape with the wells of the ultrathin-walled microwell plate.
- means for heating and cooling the sample block comprising at least one thermoelectric pump
- means for sealing the plurality of samples comprising a high-pressure heated lid.

[0005] The invention is more specifically illustrated by the accompanying figures:

Figure 1 illustrates the diagram of the ultrathin-walled microwell plate
Figure 2 illustrates the diagram of the rapid heat block thermocycler.
Figure 3 illustrates the photograph of an agarose gel the DNA in which was visualized by ethidium bromide and UV light. This photograph demonstrates rapid amplification of a 454-bp fragment of human papillomavirus (HPV) DNA.

[0006] The first and major aspect of the present invention concerns the use of small, low-profile, ultrathin-walled multiwell plates for holding small biological samples (i.e. 0.5-10 µl) (1). Especially important is the considerably decreased thickness (i.e. 10-20 fold) of the well walls compared to conventional, thin-walled PCR plates. This can be reached, for example, rather by means of thermoforming thin thermoplastic films than by injection molding. An additional great advantage is that thermoforming, due to the small tooling costs, is much less expensive than high-precision injection molding which is needed to produce extremely thin parts. Such thermoplastic films are, for example, polyolefin films, such as metallocene-catalyzed polyolefin films, copolymer films and cast polypropylene films, such films having a thickness of not more than 50 µm. Preferentially, the multiwell plate is vacuumformed out of a 30-50 µm cast, unoriented polypropylene film. Usually, the film is formed into a "female" mold comprising a plurality of spaced-apart, conically shaped wells which are machined in the body of a rectangular- or square-array shaped mold. The advantage of vacuumforming into a "female" mold is that the thickness of the walls of the formed wells is gradually reduced to 15-20 µm at the bottoms of the wells. The plastic material polypropylene is compatible with the standard PCR chemistry and therefore widely used for injection molding of PCR tubes and/or multiwell plates. In addition, it has a reduced water vapor sorption when compared to other plastics (e. g. polycarbonate). The volume of the wells is not more than 40 µl, preferably 20 µl, the height of the wells is not more than 3.8 mm, the diameter of the openings of the wells is not more than 4 mm and the inter-well spacing is not more than 4.5 mm. Usually the number of wells is in the range of 36-96. As shown in Figure 1, the handling of the plate (1) containing the multiple wells (2) is facilitated, by a rigid 0.5-1 mm thick plastic frame (3) which is heat bonded to the plate. The thickness of the well walls of the film-formed plate is reduced 10-20 fold when compared to the conventionally injection-molded PCR plates. By means of the well known Fourier equation for the heat transfer in solid substances, it can be shown that the heat transfer through the walls of the film-formed plates is 10-20 fold faster when compared to conventional PCR tubes. In practice, the temperature of small samples (1-10 µl) contained in ultrathin-walled plates equilibrates with the temperature of the sample block (4, see Figure 2) in 1-3 seconds. For comparison, it takes 20-40 seconds to equilibrate the temperature of 10-100 µl samples with the temperature of the sample block when the samples are contained in conventional thin-walled PCR tubes. The other principal advantage of the use of low-profile plates with relatively large openings of the wells (i.e. a diameter of 4 mm) for rapid temperature cycling of multiple samples is that small samples can be rapidly and accurately placed into the wells by means of conventional pipetting equipment. In this case no special skills are necessary when compared to the time consuming and labor-intense loading of capillaries or microreactors.

[0007] The second aspect of the invention is, that, in order to ensure the efficient and reproducible sealing of small samples (5, this and the following numbers refer to Figure 2) by using heated-lid technology, the conically shaped wells (2) are of identical height with similarly shaped wells machined in the body of the sample block (4) of the thermocycler. In contrast to conventional PCR plates, the ultrathin -walled plate closely fits the array of the sample wells and the top surface of the sample block (4). Thus, as shown in Figure 2, the geometry of the wells enables the positioning of the entire multiwell plate (1) into the sample block (4). In this case the pressure caused by a screw mechanism (6) of the heated lid is actually directed to those parts of the multiwell plate which are supported by the top surface of the sample block (4) and not to the thin walls of the wells of the plate as it is the case for the PCR tubes or conventional PCR plates (see US Patent No 5,475,610). This advantage makes it possible to increase the sealing pressure of the heated lid several fold (i.e. 5-10 fold) compared to the conventionally used pressure of 30-50 g per well without cracking the conically shaped walls. The tight thermal contact between the extremely thin walls of the wells and the body of the block (4) is achieved automatically by the increased air pressure arising in the sealed wells at elevated temperatures. The high pressure heated lid comprises a screw mechanism (6), a heated rigid metal (e.g. aluminium) plate (7) and an elastic insulating gasket (8). Conventionally, the metal plate (7) is heated by resistive heating, its temperature is sensed by a thermistor (9) such as a commercially available one, and controlled by a programmable controller (10). The elastic gasket (8) is usually a 1.5-2 mm thick silicon-rubber gasket. It serves for a tight pressuring of the sealing film (11) made of, for example, polypropylene, to the top surface of the multiwell plate (1) and for the thermal isolation of the sample block (4) from the metal plate (7). The sealing film (11) is usually a 50 µm thick polypropylene film. It prevents the contamination of the gasket (8) by PCR products during thermocycling. Surprisingly, by the above means of sealing the plates, samples of a volume of as few as, for example, 0.5 µl can be easily amplified without reducing the PCR efficiency.

For comparison, conventional, low-pressure heated lids can be reliably used for oil-free temperature cycling of samples of a minimum volume of 20 µl (See US Patent No 5,475,610).

[0008] The third aspect of the invention concerns the use of a low profile, low thermal capacity metal-sample block (4), e.g. of silver (or silver alloy) or of oxygen-free copper, for holding the multiwell plates. The sample block has a major top surface and a major bottom surface. An array of spaced-apart sample wells is formed in the top surface of the block. Usually the height of the 3cm x 3cm (or 4cm x 4cm) block is not more than 4 mm and the thermal capacity is of approximately 3.5 to 7

watt-seconds per °C. This allows the use of a standard, 40-90 watt thermoelectric pump (Peltier module) (12) for rapid (greater than 5° C per second) heating and cooling of the sample block. For comparison, the sample block for holding 96 conventional PCR tubes has the thermal capacity of 500-600 watt-seconds per °C (see US Patent No 5,475,610). The average heating-cooling rate of conventional heat block thermocyclers is usually limited to 1-2.5° C per second because of the high thermal capacity of the sample blocks. A single thermoelectric module for heating and cooling has the advantage of an improved thermal contact between the module (12) and the sample block (4) and the module and the air-cooled heat sink (13), e.g. of aluminium, when compared to the use of multiple modules due to the height differences between the modules. The thermocouple (14), e.g. the copper-constantan thermocouple, (each wire: about 50 µm diameter) with a time constant not greater than 0.01 sec is used for sensing the temperature of the sample block (4). The programmable controller (10) is used for a precise time and temperature control of the sample block (4). Using the metal multi sample block identical thermocycling conditions are guaranteed for all samples of one PCR run. This is in contrast to individual microreactors or capillaries, where a uniform heating and cooling of multiple samples cannot be guaranteed.

[0009] Summarized, this invention has many advantages when compared to capillary or microfabricated rapid thermocyclers. Multiple small-volume samples can be easily loaded into the wells of ultrathin-walled multiwell plate by conventional pipetting equipment. Furthermore, they can be rapidly and efficiently sealed by using a high-pressure heated lid. Upon amplification the samples can be easily recovered for product analysis by electrophoresis or hybridization, thus allowing also high throughput amplification. Finally, standard PCR mixtures can be used for rapid temperature cycling without adding carriers, like BSA. Last but not least, the use of disposable, inexpensive, ultrathin-walled plates allows a great reduction of the total costs.

[0010] The following examples serve to illustrate the invention but should not be construed as a limitation thereof.

Example 1:

[0011] A heat block thermocycler for subjecting a plurality of samples to rapid thermal cycling according to the invention is depicted in Fig. 2, wherein

3) is a 0.5 mm thick plastic frame with a 50 µm thick polypropylene multiwell plate
4) is the 3 cm x 3 cm sample block (oxygene-free copper)
5) is a 3 µl sample
6) is the screw mechanism of the heated lid
7) is the heated aluminium plate (thickness: 4mm)
8) is the elastic insulating, 1.5 mm thick silicon-rubber gasket
9) is the thermistor
10) is the programmable controller
11) is the 50 µm thick polypropylene sealing film
12) is the 40 watt, thermoelectric pump (3 cm x 3 cm; Peltier module)
13) is the air-cooled aluminium heat sink (15 cm x 15 cm x 4 cm)
14) is the copper-constantan thermocouple (each wire: 50 µm diameter).

Example 2:

[0012] Figure 3 illustrates the photograph of an electrophoretically separated 454-bp fragment of human papillomavirus DNA. The fragment was amplified by using the rapid heat block thermocycler according to Example 1 operating at the average ramping rate of 4.5° C/second and 50 µm thick polypropylene multiwell plate. The conditions for the reaction mixture were as described by Ting and Manos (PCR protocols, chapter 42 (1990) Eds.: Innes, Gelfand, Sninsky and White, ISBN 0-12-372180-6), except that a sample volume of 3 µl was used. Incubation times were as follows: denaturing: 3 seconds at 95° C, annealing time: 3 seconds at 55°C, extension time: 16 seconds at 72° C, number of cycles: 30; total amplification time: 20 minutes. Line 1-5: samples (10^4 of input viral DNA copies) placed randomly into wells of a 36-well plate. Line 6: molecular weight marker (Lambda-phage DNA, pstI-restriction digest).
As it can be seen from the figure, the product yield and specificity of the exponential DNA amplification reaction was very high, although the total amplification time was 20 minutes only and the reaction was performed in excess of human genomic DNA.

Claims

1. A heat block thermocycler for subjecting a plurality of samples to rapid thermal cycling, the heat block thermocycler comprising

   - means for holding the plurality of samples comprising
     - the ultrathin-walled multiwell plate capable of holding a plurality of samples, and
     - a low profile, low temperature capacity sample block having an array of wells, wherein the said wells are of similar height and shape with the wells of the ultrathin-walled microwell plate.
   - means for heating and cooling the sample block comprising at least one thermoelectric pump
   - means for sealing the plurality of samples comprising a high-pressure heated lid.

2. A heat block thermocycler according to claim 1 wherein the said ultrathin-walled multiwell plate has a wall thickness of not more than 50 μm.

3. A multiwell plate according to claim 2 wherein the said plate comprises an array of wells of a volume of not more than 40 μl.

4. A heat block thermocycler according to claim 1 wherein the said sample block has a height of not more than 4 mm.

5. A sample block according to claim 4 wherein the said block has a thermal capacity of not more than 6 watt seconds per °C.

6. A heat block thermocycler according to claim 1 wherein the temperature of the said sample block can be rapidly and controllably increased and decreased at a rate of at least as great as 5 °C per second by a single thermoelectric pump.

7. A heat block thermocycler according to claim 1 wherein the high pressure heated lid has an elastic insulating gasket.

8. A heated lid according to claim 7 wherein the elastic insulating gasket is a silicon-rubber gasket.

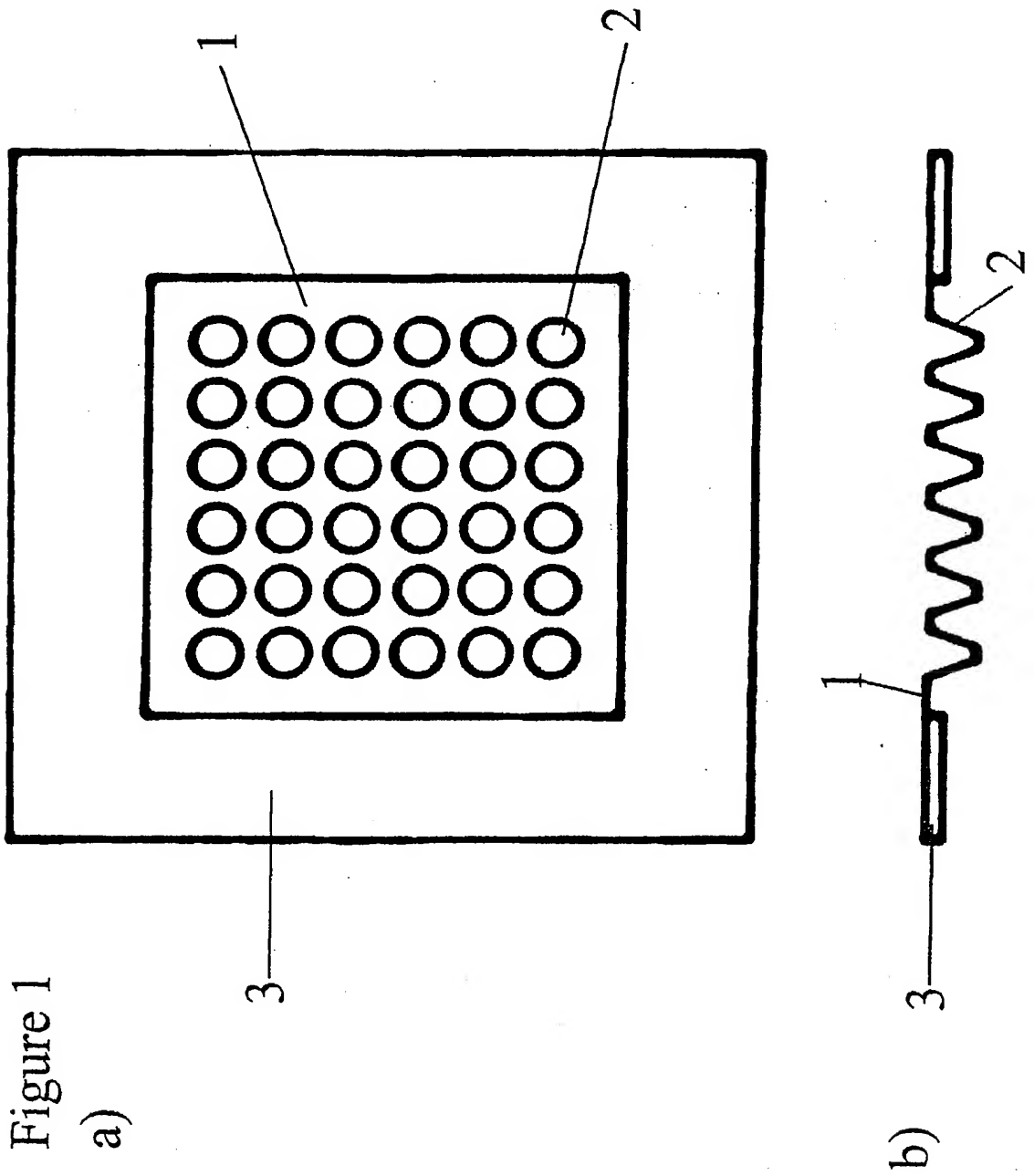


Figure 1

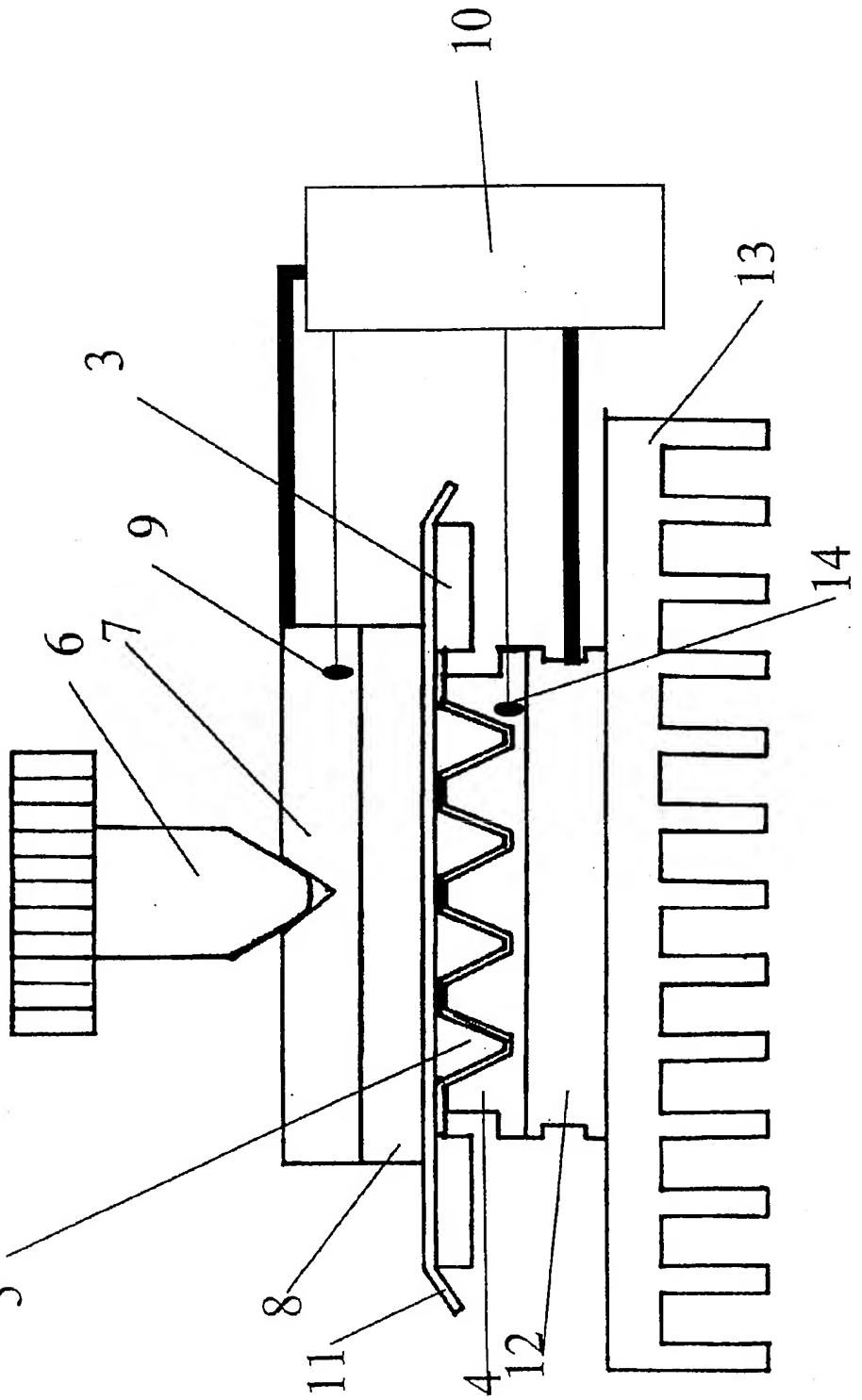
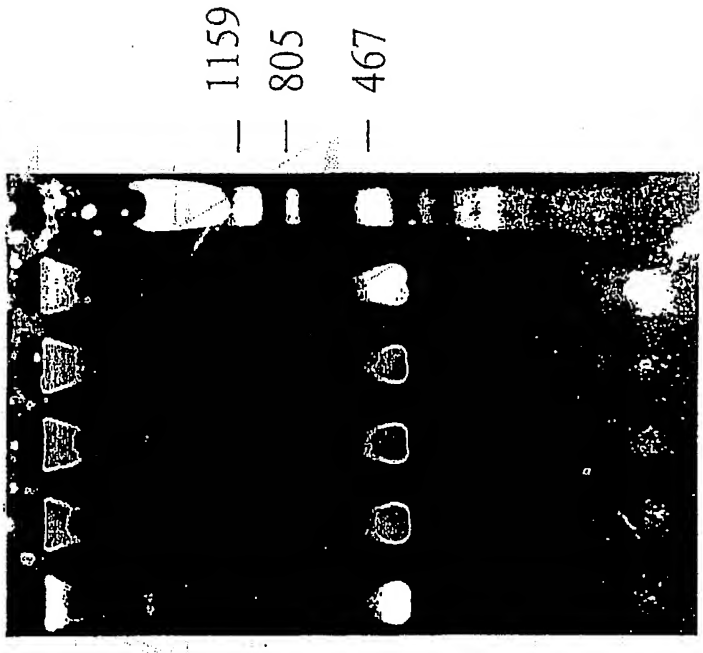


Fig. 2

Figure 3



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EUROPEAN SEARCH REPORT

Application Number
EP 99 10 6900

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION
Y	WO 98 43740 A (HETHERINGTON PAUL M ;NOREIKS RICHARD W (US); PERKIN ELMER CORP (US) 8 October 1998 (1998-10-08) * page 3, paragraph 1 - paragraph 3; claims 1-7 *	1,2,7,8	C12Q1/68 B01L7/00
Y	* page 6, paragraph 3 - page 7, paragraph 1 *	1,2	
Y	* page 14, last paragraph - page 15, paragraph 2 *	1,7,8	
A	* page 15, paragraph 3 - page 16, paragraph 1 *	6	

Y	DE 40 22 792 A (MAX PLANCK GESELLSCHAFT) 6 February 1992 (1992-02-06) * column 3, line 22 - line 28 *	1,2	
A	* column 8, line 25 - line 44 * * column 17, line 11 - line 23; figures 4,10 *		

Y	US 5 508 197 A (HANSEN ANTHONY D A ET AL) 16 April 1996 (1996-04-16) * column 7, line 64 - column 8, line 4; figures 2,4 * * column 8, line 54 - line 60 * * column 9, line 40 - line 60; figures 2,4 *	1,7,8	TECHNICAL FIELDS SEARCHED B01L

A	US 5 496 517 A (PFOST ROBERT F ET AL) 5 March 1996 (1996-03-05) * column 4, line 21 - column 5, line 19; figures 5,6 *	1-8	

A	US 5 802 816 A (DIETZEL GUENTER) 8 September 1998 (1998-09-08) * column 3, line 50 - column 4, line 24; figures * * column 3, line 1 - line 13 *	2-4	

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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 September 1999	Hocquet, A

CATEGORY OF CITED DOCUMENTS

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Application Number
EP 99 10 6900

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION
D,A	US 5 455 175 A (WITTWER CARL T ET AL) 3 October 1995 (1995-10-03) * column 11, line 2 - line 27; figures 8A,8B * * column 12, line 16 - line 42; figure 8C *	1,3	
A	* column 14, line 14 - line 65 *	6	

TECHNICAL FIELDS SEARCHED

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14 September 1999	Hocquet, A

CATEGORY OF CITED DOCUMENTS

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14-09-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9843740 A	08-10-1998	AU 6786198 A	22-10-1998
DE 4022792 A	06-02-1992	AT 125732 T	15-08-1995
		WO 9201513 A	06-02-1992
		DE 59106171 D	07-09-1995
		EP 0539369 A	05-05-1993
		JP 6500727 T	27-01-1994
US 5508197 A	16-04-1996	NONE	
US 5496517 A	05-03-1996	CA 2031912 A	23-06-1991
		DE 69027015 D	20-06-1996
		DE 69027015 T	19-09-1996
		EP 0438883 A	31-07-1991
		JP 4208866 A	30-07-1992
		US 5552580 A	03-09-1996
US 5802816 A	08-09-1998	DE 4424112 A	11-01-1996
		WO 9601692 A	25-01-1996
		JP 9505008 T	20-05-1997
US 5455175 A	03-10-1995	US 5935522 A	10-08-1999

EPO FORM P0459

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